This influx of information provides new opportunities for understanding the chemistry of these proteins.
Using a structural bioinformatics approach, we have determined a strong asymmetry in the charge distribution of these proteins. For the outward-facing amino acids of the beta barrel within regions of similar amino acid density for both membrane leaflets, the external side of the membrane contains more than three times the number of charged amino acids as the internal side of the membrane. Moreover, the lipid bilayer of the outer membrane is asymmetric, and the overall preference for amino acid types to be in the external leaflet of the membrane correlates roughly with the hydrophobicity of the membrane lipids. This preference is demonstrably related to the difference in lipid composition of the external and internal leaflets of the membrane. The charge asymmetry of proteins in the outer membrane has important implications for how we understand the mechanism of outer membrane protein insertion.

1215-Pos Board B166
A Fundamental Force Governing Protein Self-Assembly in Membranes
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Using coarse-grained molecular dynamics simulations, we demonstrate the nature of a proposed phenomenon that governs self-assembly of inclusions within a lipid bilayer, inspired by the statistical mechanics of the hydrophobic effect. We study the nature of this effect on membrane inclusions of various chemistries and sizes. We identify the range of hydrophobic thicknesses over which this phenomenon occurs and characterize the effects of the proposed phenomenon on small inclusions such as cholesterol versus larger, multidomain transmembrane proteins. Our results show that this effect can provide a force for assembly and reorganization in a lipid bilayer based on the in-plane size and hydrophobic thickness of the inclusion, and the melting temperature of the surrounding lipids. We propose that this effect provides a physical framework that can explain lipid raft formation.

1216-Pos Board B167
Comparing Lo/Ld Membrane Thickness Mismatch and Miscibility Transition Temperatures using Fluorescence and Atomic Force Microscopy
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Micron-scale coexisting liquid phases appear in lipid bilayers composed of, at minimum, a ternary mixture of lipids and sterols. Lipids that are predominantly found in the liquid ordered membrane phase (the Lo phase as opposed to the liquid disordered Ld phase) feature long, saturated carbon chains that pack with high order at high areal densities [Veatch *et al.BJ* 2004]. Membrane thickness is an especially interesting parameter due to its effects on the trafficking and function of membrane proteins [Simons and Sampaio, *Cold Spring Harb. Perspect. Biol.* 2011]. Experimental results [García-Sáez *et al. J. Biol. Chem*. 2007] present the trend that miscibility transition temperatures increase with increasing thickness difference between the domains and the surrounding membrane. Here we test the robustness of this result by applying the same techniques (fluorescence microscopy and room temperature atomic force microscopy) to different lipid mixtures than those studied by García-Sáez *et al*.

1217-Pos Board B168
The Effects of Walp Peptides on Phase Behavior in Quaternary Lipid Mixtures: A Molecular Dynamics Study
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Quaternary lipid mixtures containing a high-melting lipid, a nanodomain-inducing low-melting lipid, a macrodomain-inducing low-melting lipid and cholesterol reveal behaviors not observed in ternary mixtures. Through fixing the amounts of high-melting lipid, total low-melting lipid and cholesterol and altering only the relative amounts of the two low-melting lipids, domain size in quaternary mixtures can be finely tuned from nanoscopic to macroscopic, with an intermediate patterned phase morphology. We have previously used extensive coarse grain and atomistic molecular dynamics simulations to study one such quaternary lipid mixture, containing the high-melting lipid DPPC (16:0,16:0-PC), the low-melting lipids PUPC (16:0,18:2-PC) and DUPC (18:2,18:2-PC), and cholesterol. In particular, we quantified the effect of the two low-melting lipids on domain size, alignment, lipid order and lipid tilt. Using those simulations as a control, we are currently evaluating how adding WALP peptides to the quaternary mixture affects the sizes, alignment and properties of coexisting phases using coarse grained molecular dynamics. We examine how these properties are affected by both the length of the WALP peptide as well as their concentration. A main focus of our work is analyzing the extent to which the WALPs alter the onset of large-scale phase separation and domain alignment. The addition of WALP to the quaternary systems makes these simulations some of the most complex and biologically relevant membranes studied to date with molecular dynamics.

1218-Pos Board B169
Appearance of Modulated Bilayer Morphology for Coexisting LD and LO Phases is Correlated with Line Tension
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Giant Unilamelar Vesicles (GUVs) appear uniform for the brain-SM/POPC/Chol bilayer mixture, whereas GUVs exhibit macroscopic domains of coexisting liquid-disordered + liquid-ordered phases for the brain-SM/DOPC/Chol mixture. We can travel through composition space in order to study this transition from nanodomains with POPC to macrodomains with DOPC. Using ρ defined as the ratio [DOPC]/[DOPC]+[POPC], the domain morphology undergoes a transition regime where ''modulated phases'' appear as a function of ρ. The formation of these different morphologies on a GUV can be understood as a competition between line tension, which favors large domains, and bending energy, which favors small domains (Amazon et al). We measured the ρ values where modulated phases appear for different 4-component mixtures, using brain-SM, or egg-SM, or palmitoyl-SM as the high-melting lipid, and DOPC/POPC or DOPC/SOPC as the low melting lipids, for a total of six different 4-component mixtures. We then measured the line tension of the macroscopic domains vs ρ, and found the same line tension at ρ values at the transition between modulated phases and macroscopic phases. This finding implies that line tension has major control over domain morphology. We are currently studying how the transmembrane peptide, GWALP-23, changes the morphology by changing the values of the competing interactions.

1219-Pos Board B170
Membrane Bending Modulus for Ternary Mixture Models of the Cell Plasma Membrane
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Ternary mixtures of high-melting lipid, low-melting lipid, and cholesterol exhibit a region of liquid-ordered + liquid-disordered phase coexistence analogous to raft + non-raft behavior in cells. These coexisting phases manifest domain sizes that range from a few nanometers to many microns, depending strongly on the nature of the low-melting lipid. When POPC, which gives rise to nanodomains, is replaced by DOPC, which yields macrodomains, an intermediate region is observed of patterned, or modulated phases. This domain morphology can be explained as a competition between line tension and bending energies with patterns occurring when the two are nearly balanced. Necessary for testing this model are measurements of line tension, and bending moduli for both phases. Here we report the bending moduli of coexisting Lo and Ld phases from mixtures that produce domains ranging in size from nanoscopic to macroscopic. Measurements were made by shape analysis of giant unilamellar vesicles with both fluorescence and phase contrast microscopy. Vesicles of a single phase were made by the gentle hydration method to obtain a more narrow distribution of vesicle tensions than is obtained by electroformation. A transmembrane helical peptide, WALP23, strongly partitions to the liquid disordered phase, and changes the size of coexisting Lo + Ld domains. We are currently investigating the effects of WALP23 on membrane mechanical properties and line tension.

1220-Pos Board B171
Minor Changes in Sterol Structure Impact the Miscibility Temperatures of Model Cell Membranes Significantly
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Evolution selected for an array of structurally similar sterols in cell membranes. While mammalian cells rely on cholesterol, yeast and fungi utilize ergosterol and plants incorporate a variety of phytosterols. Although the chemical structure of ergosterol and phytosterols differ only slightly from cholesterol, these small differences have been shown to significantly impact the solubility limit of the sterols in model cell membranes [Stevens & Keller, 2010, 5882]. In our current study, we use fluorescence microscopy to correlate the structural features of ergosterol, stigmasterol, and ß-sitosterol with the miscibility temperature of ternary membranes containing DOPC, DPPC, and a sterol. We map the full ternary phase diagram of each system and compare it with that of the well-characterized DOPC/DPPC/cholesterol system. Our data provides insight into the intermolecular interactions taking place between sterols and PC-lipids and reveals that seemingly minor changes in sterol structure have a large impact on membrane miscibility temperatures.